The Application of Through Vial Impedance Spectroscopy (TVIS) for Process Parameter Determination in Freeze-Drying Method Development

PDA Europe
Pharmaceutical Freeze Drying Technology
Strasbourg, 27 – 28 September 2016
Introduction of Through Vial Impedance Spectroscopy (TVIS) Technology

TVIS Applications

- $K_V$ Determination
- $R_P$ Determination
- Other Applications
  - Ice nucleation,
  - Glass transition,
  - Eutectic Crystallization
  - Collapse
Through Vial Impedance Spectroscopy (TVIS)
• Impedance measurements across a vial rather than within the vial
• Hence “Through Vial Impedance Spectroscopy”
• “Non-product invasive”
• Stoppering possible
• Non-perturbing to the packing of vials

• On going TVIS development for multiple scales:
  ➢ Product Development (Microplate, Micro-vial, or Single vial)
  ➢ Mini-Pilot (Small population clusters of vials)
  ➢ Scale up to Batch (Large population cluster of vials)

• Mesoscale accessible by assessing the temperature dependence of impedance
Freeze Dryer and TVIS System

Virtis Advantage Pro Freeze Dryer

TVIS System

Through Vial Impedance Spectroscopy (TVIS)

Junction box

Pass-through

Impedance test vial
Electrodes are attached to the external surface of a vial.
- The vial is filled with the sample to fill factor (Φ) of 0.9.

\[
\text{Fill factor (Φ)} = \frac{\text{The height of liquid fill}}{\text{The height of active electrode}}
\]

- The sample has both resistive & capacitive properties.
- The glass vial has both resistive & capacitive properties.
- But glass resistance is so high therefore it behaves primarily as a capacitor.
- In freeze drying, the sample is frozen and dried by sublimation to obtain a ‘dry’ layer.
- The dry layer is predominantly capacitive due to its low moisture content / high resistance.

Exception - Proteins
Impedance is a frequency dependent parameter largely because the impedance of a capacitance is dependent on the frequency of the applied field, whereas an ideal resistor has zero frequency dependence.

In the boxes show small impedance spectrum of \( \log |Z| \) vs \( \log f \) for a capacitor and another spectrum for a resistor, then show a spectrum with \( R \) and \( C \) in parallel.

By fitting the impedance spectrum one can extract the sample resistance and capacitance.
• At $\omega \to 0$, $C'' = 0$

• As the frequency increases, $C''$ increases to maximum ($C''_{max}$) then decreases to 0 as the frequency $\omega \to \infty$

• At a frequency of

$$\omega_{max} = \frac{1}{R_s(C_S+C_G)} \text{ in radians}$$

$$f_{max} = \frac{1}{2\pi R_s(C_S+C_G)} \text{ in cycles per second}$$

$$C''_{max} = \frac{C_G^2}{2(C_S+C_G)}$$
Annealing = Re-heating and Re-cooling
Freezing Process

Primary Drying Process

TVIS response surface

Freezing

C' / pF vs. Frequency (Hz)

-24.3 °C
-5.7 °C
12 °C
24.1 °C

C'' / pF vs. Frequency (Hz)

-24.3 °C
-5.7 °C
12 °C
24.1 °C

Frequency (Hz)

C' / pF

C'' / pF
The magnitudes of $C_G$ and $C_S$ are proportional to the area of interface between the frozen mass and the glass adjacent to the electrode ($A$).

$C_G = \frac{\varepsilon_G \varepsilon_0}{d_G} A$

Constant ($K_G$)

$C_S = \frac{\varepsilon_S \varepsilon_0}{d_S} A$

Constant ($K_S$)

$C''_{max1} = \frac{C_G^2}{2(Cs + CG)} = \frac{(K_G A_1)^2}{2(K_S A_1 + K_G A_1)} = kA_1$

$C''_{max2} = \frac{C_G^2}{2(Cs + CG)} = \frac{(K_G A_2)^2}{2(K_S A_2 + K_G A_2)} = kA_2$

In case of a flat sublimation interface, the interfacial area between the frozen layer and the juxtaposed glass wall ($A$) will decrease in proportion to the remaining ice volume.
The capacitance spectrum is dependent on both the electrical resistance and electrical capacitance of the vial contents.

- Data viewing software (LyoView™) identifies the peak frequency ($F_{PEAK}$) and peak amplitude ($C''_{PEAK}$) in the imaginary part of the capacitance spectrum.

- In general terms:
  - $F_{PEAK}$ can be used to monitor phase behaviour (ice formation, glass transitions) and product temperature.
  - $C''_{PEAK}$ can be used to monitor the amount of ice remaining during primary drying, from which the drying rate and end point may be determined.
TVIS Application
Heat Transfer Coefficient ($K_v$) Determination
(*Test sample: 2 mL water in 10 mL vial*)
Primary Drying Modelling: Heat and Mass Transfer

\[
\frac{dm}{dt} = \left( \frac{P_{ICE} - P_{CHAMBER}}{\hat{R}_p} \right) \cdot A_P
\]

\[
\frac{dq}{dt} = L \left( \frac{dm}{dt} \right)
\]

K\textsubscript{V} determination

\[
\frac{dq}{dt} = A_V K_V (T_{SHELF} - T_{PRODUCT})
\]

\[
\ln(PICE) = \left( \frac{-6144.96}{T_{PRODUCT}} \right) + 24.02
\]

\[
\frac{dm}{dt} - \text{sublimation rate (g/hour/vial)}
\]

\[
P_{ICE} - \text{Vapour pressure at ice sublimation interface (Torr)}
\]

\[
P_{CHAMBER} - \text{Chamber pressure (Torr)}
\]

\[
\hat{R}_p - \text{Area normalized product and stopper Resistance}
\]

\[
\frac{dq}{dt} - \text{heat flow to the product}
\]

\[
L - \text{Latent heat of sublimation (J g}^{-1})
\]

Heat Transfer Coefficient ($K_V$) Determination

- The product temperature ($T_{PRODUCT}$), which derived by TVIS as $T(F_{PEAK})$ or by thermocouple ($TC$), is one of parameters needed for $K_V$ determination.

- Sublimation rate ($\frac{dm}{dt}$) is estimated by TVIS.

- Test sample is 2 mL water filled in 10 mL vial.

Temperature Calibration

Temperature Calibration

$$y = -10.97x^2 + 99.202x - 229.59$$

Calculate $T_{PRODUCT}$ from Log $F_{PEAK}$ (TVIS).

Primary drying

$0.244 \text{ pF} \equiv 2 \text{ g water}$

Drying rate ($\frac{dm}{dt}$) $\sim 0.3 \text{ g/h}$

$T_{PRODUCT}$ and $\frac{dm}{dt}$ from TVIS

$K_V$ determination
First convert \( \frac{dm}{dt} \) to \( \frac{dq}{dt} \) using the latent heat of sublimation \((L = 2844 \text{ J g}^{-1})\)

\[
L \frac{dm}{dt} = \frac{dq}{dt}
\]

\[
dm/dt = 0.3 \text{ g/h}
\]

\[
L = 2844 \text{ J g}^{-1}
\]

\[
dq/dt = 853 \text{ J/h}
\]

\[
\frac{dq}{dt} = A_v K_v (T_{\text{SHELF}} - T_{\text{PRODUCT}})
\]

\[
T_{\text{SHELF}} = -20 \text{ °C}
\]

\[
T_{\text{PRODUCT}} = -34.2 \text{ °C}
\]

\[
A_v = 0.0045 \text{ m}^2
\]

(\text{Schott Type1 glass 10 ml tubing vial})

\[
K_v = 37 \text{ W m}^{-2} \text{ K}^{-1} \; [@ \; 40 \text{ Pa}, \; 400 \mu\text{Bar}]
\]

TVIS Application

Heat Transfer Coefficient ($K_v$) Determination

(*Test sample: 2 mL protein solution fill in 10 mL vial*)
The different types of resistance to mass transport (water vapour flow)

\[
\frac{dm}{dt} = \left( \frac{P_{ICE} - P_{CHAMBER}}{R_p + RS + NRC} \right)
\]

Product resistance \((R_p)\) is dominated around 80% of the mass transfer therefore the other resistances could be ignored (Pikal, Roy and Shah (1984)).

\[
\frac{dm}{dt} = \left( \frac{P_{ICE} - P_{CHAMBER}}{\hat{R}p} \right) \cdot A_p
\]

\(\frac{dm}{dt}\) = the rate of mass transfer for the water vapour or sublimation rate (g/hour/vial)

\(P_{ICE}\) = the equilibrium vapour pressure of ice at the sublimation interface temperature (Torr)

\(P_{CHAMBER}\) = the chamber pressure (Torr)

\(\hat{R}p\) = the area normalized resistance of the dried product (cm\(^2\)·Torr·h·g\(^{-1}\))

\(A_p\) = the cross-sectional area of the product (cm\(^2\))

**Preparation: 50% v/v Protein Solution**

- A 50% v/v protein solution 2 mL has protein 0.042 g
- Protein 1 g has unavailable water to colligative effect of ~0.3 g (unfrozen water)
- Therefore unfrozen water of protein is 0.3 x 0.042 = 0.0126 g
- The weight of ice is approximately 2 - 0.0126 = 1.974 g

**Freeze drying Protocol**

- Apply Vacuum

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PBS 16 mL

4.2% w/v Protein Solution 16 mL

50% v/v Protein Solution 2 mL
The peak at high frequency (\(~12 \text{ kHz}\)) is due to the ice layer:
- Ice layer peak decreases during the early stages of primary drying (< 25 h) because the peak amplitude is proportional to the volume of the ice layer.

The peak at low frequency is due to the dry layer:
- Dry layer peak increases during the later stages of primary drying (> 27h) because the peak amplitude is proportional to the volume of the dry layer.
Dried Product Resistance ($R_P$) Determination of Protein Solution

\[
\frac{dm}{dt} = \left( \frac{P_{ICE} - P_{CHAMBER}}{\hat{R}_P} \right) \cdot A_P
\]

Ice vapour pressure calculated from $T_{PRODUCT}$ the product temperature (as derived by TVIS or Thermocouple)

\[
\hat{R}_P = \left( \frac{P_{ICE} - P_{CHAMBER}}{\frac{dm}{dt}} \right) \cdot A_P
\]

\[
\ln(P_{ICE}) = \left( \frac{-6144.96}{T_{PRODUCT}} \right) + 24.02
\]

\[
\ln(P_{CHAMBER}) = \left( \frac{-6144.96}{T_{CONDENSER}} \right) + 24.02
\]

$dm/dt$ can estimate by TVIS

Chamber pressure calculated from the temperature of condenser ($T_{CONDENSER}$) (in case of condenser is in the freeze dry chamber)

Cross-sectional area of product can calculate by using internal diameter of tested vial ($A = \pi r^2$). For example, Schott Type1 glass 10 ml tubing vial has internal diameter 2.2 cm therefore, $A_P = 3.8 \text{ cm}^2$
The drying rate \( \frac{dm}{dt} \) estimated by TVIS is one of parameters used for determination of dried product resistance \( R_P \).

First convert \( C''_{\text{PEAK}} \) from TVIS to Ice mass:

\[ C''_{\text{PEAK}} \text{ is proportional to the amount of ice} \]

Then, calculate drying rate \( \frac{dm}{dt} \) using time derivative of ice mass:

\[ y = 0.0009x^2 - 0.0921x + 2.5341 \]

\[ \frac{dm}{dt} = 0.0018x - 0.0921 \]

\( R_P \) determination
Dried Product Resistance ($R_P$) Determination of Protein Solution

\[
\ln(P_{ICE}) = \left(\frac{-6144.96}{T_{PRODUCT}}\right) + 24.02
\]

\[
\ln(P_{CONDENSER}) = \left(\frac{-6144.96}{T_{CONDENSER}}\right) + 24.02
\]

\[A_p = 3.8 \text{ cm}^2\]

(Schott Type1 glass 10 ml tubing vial)

\[\frac{dm}{dt}\]

\[\hat{R}_P = \left(\frac{P_{ice} - PC}{\frac{dm}{dt}}\right) \cdot A_p\]
New slide showing the relationship between ice remaining and dry layer thickness
Dried Product Resistance ($R_p$) Determination of Protein Solution

$R_p$ (cm$^2$·Torr·h·g$^{-1}$) vs. Dry Layer Thickness (cm)

- 5% BSA annealing 1 h (ref. 2)
- 2% BSA annealing 1 h (ref. 2)
- 2% BSA non annealing (ref. 3)
- 2% IgG non annealing (ref. 3)
- 2% Lysozyme non annealing (ref. 3)
- 5% Glycine non annealing (ref. 1)
- 0.5% BSA annealing 6 h (ref. 2)
- 4% Protein solution annealing 1 h
- 4% Protein solution annealing 1 h

Summary & Future Work

$K_v$ value determination possible.
- Increase the drying time over which $T(\text{Feak})$ maybe determined by increasing the fill volume of ice.
- Investigate the dependence on chamber pressure for design space determination

$R_p$ value determination possible:
- Investigate uncertainties in temperature measurement to provide for more reliable estimates of $R_p$
- Use equivalent circuit modelling to fit spectra and develop a model for a TVIS surrogate for temperature
- Characterise a wide range of materials, formulations and process parameters, inc. nucleation temperature, fill volume, freezing rate, annealing
TVIS Application

Other Applications
Product characterization: Ice Nucleation

Log $F_{\text{PEAK}}$ profile of freezing step shows the ice nucleation process:

- Solution is cooled at A
- Solution super-cools and starts ice nucleation at B. This point is referred as “onset of ice nucleation”
- Crystallization or ice nucleation formation occurs during C period and continues until the end point of solidification at D.
Product characterization: Glass transition, Eutectic Crystallization, Collapse

Glass transition of 10% maltodextrin DE16-19

-19±0.5 °C by DSC

- 17 °C by DSC

Start of T ramp

2nd time derivative of C''

A suddenly change in the capacitance demonstrates the collapse of cake


Suppression of mannitol eutectic by sucrose

Ice

S/M

M
Summary of Applications of TVIS

• TVIS registers thermal events through changes in the sample resistance associated with
  - Discontinuous changes in viscosity (glass transition, collapse)
  - Change of state (e.g. ice formation and eutectic formation)
• Temperature control might be possible through improved modelling and calibration (Equivalent circuits)
• Primary drying (loss of ice) is monitored through changes in the strength of the dielectric loss peak (or step in the real part capacitance)
  - Enables drying rate determinations for \( K_v \) and \( R_p \) calculations
• Meso-structural information extracted through the (non-Arrhenius) temperature dependence of the resistance
• Mechanisms of annealing may be elucidated from changes in resistance with time (during the heating-hold phase) and from the absence of any changes in \( T_G \)
• Future (with non-contact system)
  - Opportunities to track the physical characteristics over a range of scales, from micro-titre plates to collections of vials
Recent Funding

LyoDEA
£217 160 Collaborative R&D funding
(Nov ’08-Oct ’12) Innovate UK

BioStaRT
£367 567 Collaborative R&D funding
(Aug ’14- Jul ’17) Innovate UK

AtlasBio
£803 846 Collaborative R&D funding
(Oct ’16-Sept 18) Innovate UK

IP: GB2480299
Electrical monitoring of a lyophilization process
Priority Date: 12th May 2010
Assignee: GEA Pharma
Acknowledgements

GEA Pharma Systems (Trevor Page, Julian Taylor)
BlueFrog Design (Chris Samwell, Ben Irvine, John Hall)
NIBSC (Paul Matejtschuk)
OnkoLytika Ltd (Mark Ecclestone, Annette Williams)
Genzyme Ireland (Tim McCoy)

Innovate UK for part funding this study
The impedance of the model can be calculated from the following equation

\[ Z^* = \frac{1}{i\omega C^*} = \frac{1}{i\omega C_G} + \frac{1}{\frac{1}{R_S} + i\omega C_S} \]

which re-arranges to

\[ Z^* = \frac{1}{i\omega C_G} + \frac{R_S}{1 + i\omega R_S C_S} = \frac{1 + i\omega R_S (C_G + C_S)}{i\omega C_G - \omega^2 R C_G C_S} \]

### Impedance can also be expressed in terms of a complex capacitance

\[ C^* = C' + C'' = \frac{1}{i\omega Z^*} = \frac{C_G + i\omega R_S C_G C_S}{1 + i\omega R_S (C_G + C_S)} \]
• From the complex capacitance formula, the expressions for real and imaginary capacitance can be calculated to explain the origin of interfacial polarization peak. This achieved by multiplying the nominator and denominator by the complex conjugate of the denominator and by grouping the real ($C'$) and imaginary ($C''$) parts

$$C^* = \frac{1}{i\omega Z^*} = \frac{(C_G + i\omega R_S C_S C_G)(1 - i\omega R_S (C_S + C_G))}{(1 + i\omega R (C_S + C_G))(1 - i\omega R_S (C_S + C_G))} = \frac{C_G + \omega^2 R_S^2 C_S C_S (C_S + C_G) - i\omega R_S C_G^2}{1 + (\omega R_S((C_S + C_G))^2}$$

To obtain

$$C' = \frac{C_G + \omega^2 R_S^2 C_S C_S (C_S + C_G)}{1 + \left(\omega R_S((C_S + C_G))^2\right)}$$

and

$$C'' = -\frac{\omega R_S C_G^2}{1 + (\omega R_S((C_S + C_G))^2)}$$
The value of real part of capacitance at $\omega \to 0$ is

$$C' = CG_{(l)} = f(vice)$$

And the value at $\omega \to \infty$ is

$$C' = \frac{C_SC_G}{(C_S+C_G)}$$

It follows that the step change in capacitance is

$$\Delta C' = C_G - \frac{C_SC_G}{(C_S+C_G)}$$

$$\Delta C' = \frac{C_G^2}{(C_S+C_G)}$$
• The peak height is given by

\[
C''_{\text{max}} = \frac{C_G^2}{2(Cs + CG)}
\]

• The peak frequency is given by

\[
f_{\text{peak}} = \frac{1}{2\pi Rs(Cs + CG)}
\]

\[C' = \frac{csCG}{(Cs + CG)}\]
Low frequency process may hold information on the state/properties of the protein layer, inc. progression and end point of secondary drying.
Dried Product Resistance ($R_p$) Determination of Protein Solution